TDP-43 loss-of-function causes neuronal loss due to defective steroid receptor-mediated gene program switching in Drosophila

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Abstract: TDP-43 proteinopathy is strongly implicated in the pathogenesis of amyotrophic lateral sclerosis and related neurodegenerative disorders. Whether TDP-43 neurotoxicity is caused by a novel toxic gain-of-function mechanism of the aggregates or by a loss of its normal function is unknown. We increased and decreased expression of TDP-43 (dTDP-43) in Drosophila. Although upregulation of dTDP-43 induced neuronal ubiquitin and dTDP-43-positive inclusions, both up- and downregulated dTDP-43 resulted in selective apoptosis of bursicon neurons and highly similar transcriptome alterations at the pupal-adult transition. Gene network analysis and genetic validation showed that both up- and downregulated dTDP-43 directly and dramatically increased the expression of the neuronal microtubule-associated protein Map205, resulting in cytoplasmic accumulations of the ecdysteroid receptor (EcR) and a failure to switch EcR-dependent gene programs from a pupal to adult pattern. We propose that dTDP-43 neurotoxicity is caused by a loss of its normal function.

DOI: https://doi.org/10.1016/j.celrep.2012.12.014

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Current Opinion in Pharmacology

Novel anticoagulants in the therapy of peripheral arterial and coronary artery disease

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Anticoagulant and antiplatelet drugs are used and studied in numerous trials for primary and secondary prevention of atherothrombosis since decades. The annual rate for cardiovascular morbidity and mortality is high in patients following an acute coronary syndrome and in patients with peripheral arterial disease (PAD) due to concomitant cardiac and cerebrovascular diseases. Plaque rupture and subsequent thrombosis involves activation of both platelets and coagulation factors. Therefore the combination of aspirin and warfarin to improve prevention of atherothrombosis compared to antiplatelet therapy alone was studied but could not be established due to significantly increased risk of major bleeding compared to a nonsignificant reduction in ischemic events. During the past two decades, clinical trials focused on combined antiplatelet therapies for the prevention of secondary events following acute coronary syndromes and very recently on the new oral anticoagulants in combination with antiplatelet therapy. This review discusses the role of the new oral anticoagulants such as Factor IIa (thrombin) and Factor Xa inhibitors in atherothrombosis, their pharmacological properties and recently published clinical data in secondary prevention of atherothrombotic events and potential implications for patients with PAD.

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Current Opinion in Pharmacology 2013, **13**:294–300

This review comes from a themed issue on **Cardiovascular and renal**

Edited by **Matthias Barton**

For a complete overview see the Issue and the Editorial

Available online 15th January 2013

http://dx.doi.org/10.1016/j.coph.2012.12.005

Introduction

Atherothrombosis is defined as the occurrence of both atherosclerosis and thrombosis in an artery and results in myocardial, cerebral, limb, and reno-mesenteric ischemia [1•,2–4]. Among the atherosclerotic diseases, patients with peripheral arterial disease (PAD) have an extensively large atherosclerotic burden, often coexisting coronary (CAD) and cerebrovascular disease, and have three times increased risk for myocardial infarction or stroke as patients without PAD [3,5•]. Likewise, recurrent ischemic events are as high as 10% per annum following acute coronary syndrome [6,7]. Despite antiplatelet therapy, up to 10–20% of these patients still have cardiovascular events, indicating that the underlying atherothrombotic activity is not optimally controlled by antiplatelet agents. Therefore, the combination of antiplatelet therapy with oral anticoagulants to reduce atherothrombotic events has been studied in the Warfarin Antiplatelet Vascular Evaluation trial (WAVE) for patients with PAD [8••,9]. This trial showed a nonsignificant reduction in ischemic events but a significant increase in bleeding complications (RR 3.41; 95% CI, 1.84–6.35) during an observation period of 35 months. The bleeding risk under combined antiplatelet and anticoagulant therapy is higher in PAD than in CAD without PAD. This might be attributable to different factors such as older age, vascular fragility, and higher morbidity [10,11]. However, the combined antithrombotic therapy is not recommended for atherothrombosis in general for secondary prevention in PAD [12].

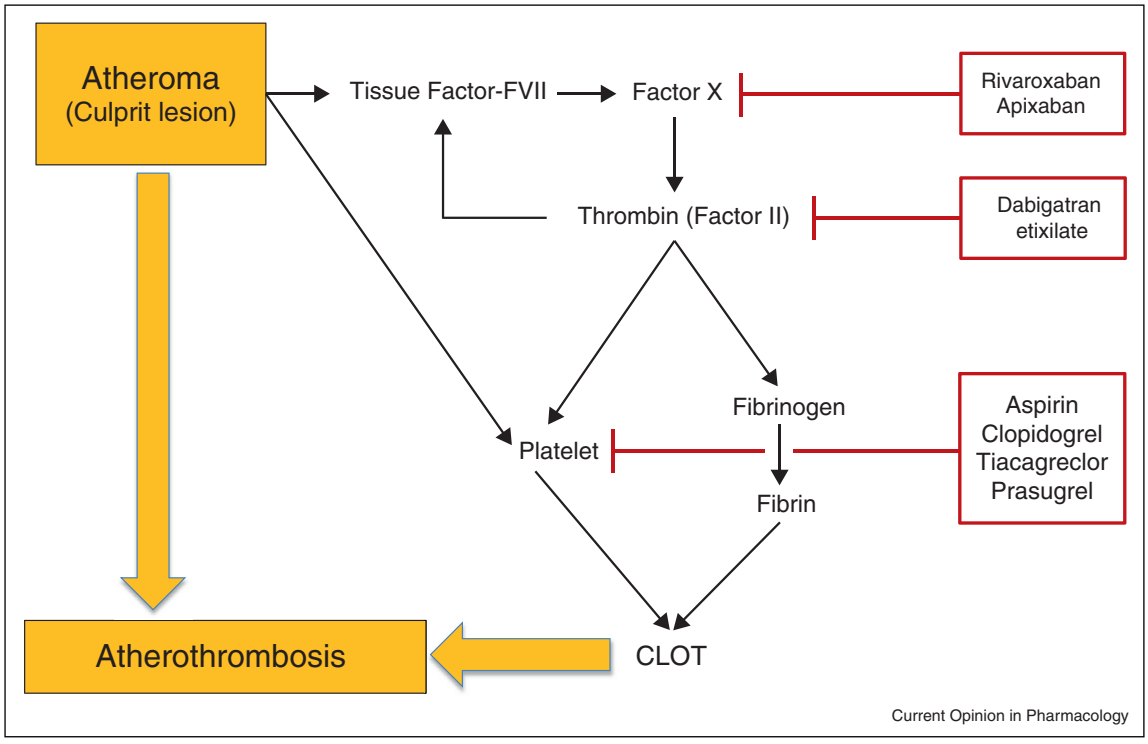
Currently in the dawn of a new anticoagulant era with several new anticoagulants that have either specific antithrombin or anti-FXa activity — in contrast to Vitamin K antagonists, that interfere in multiple ways in the coagulation cascade — more numerous trials for prevention and therapy of venous thromboembolism, prevention of embolic complication due to atrial fibrillation and therapy of atherothrombosis are reported and upcoming [13••].

Besides the afore mentioned increased risk of bleeding when combining antiplatelet drugs with Vitamin K antagonists, their narrow therapeutic windows, need for frequent laboratory monitoring, higher risk for food and drug interactions pose several disadvantages. The development of novel oral Factor Xa inhibitors and oral direct thrombin inhibitors provide an alternative to Vitamin K antagonists. In this paper, we discuss the new agents, rivaroxaban, apixaban, and dabigatran, for secondary prevention of acute atherothrombosis and their therapeutic potential for patients with PAD and CAD.

Atherothrombosis and ischemia

Thrombosis plays a critical role in the pathomechanism of ischemic syndromes, as disruption of an atherosclerotic plaque exposes blood to subendothelial collagen, tissue factor, and other procoagulant molecules such as thrombin that trigger activation of platelets and formation of

Figure 1



Simplified coagulation and clotting cascade in atherothrombosis and antithrombotic drug actions.

fibrin within the vessel lumen [14–16]. Not only endothelial damage and dysfunction but also inflammation and coagulation are closely related to the pathophysiology of ischemic syndromes [17]. Platelets play key roles in both the formation of the atheromatous plaque and clinical presentation of acute atherothrombotic events following plaque rupture. In the pathogenesis of atherothrombosis, clotting activation has a crucial role and thrombin generation is involved in both platelet activation and fibrin (Figure 1).

In animal models, hypercoagulability tends to increase atherosclerosis, whereas hypocoagulability reduces the atherosclerotic burden [18••]. Whether this direct relationship between coagulation and atherosclerosis applies for humans is not clear. Almost all coagulation proteins, including tissue factor, are found in atherosclerotic lesions in humans. In addition to generating local fibrin, an environment for cell growth, serine proteases such as thrombin are thought to be involved in cell signaling processes, acting through the activation of protease-activated receptors [17]. Activation of such protease-activated receptors on vascular cells triggers other complex processes promoting atherosclerosis, including inflammation, angiogenesis, and cell proliferation.

Direct thrombin inhibitors and anti-FXa-inhibitors are targeting at this crucial phase of thrombin generation with the potential to prevent thrombosis and progression of atherosclerosis alike. Therefore, the novel anticoagulants may have synergistic antithrombotic and bleeding balance due to their pharmacodynamic and pharmacokinetic properties which when combined with antiplatelet agents may improve overall net effects. Furthermore, combined therapy may be of benefit in aspirin resistance, which is both a clinical and laboratory problem (Kasmeridis, Apostollakis, Lip: aspirin and aspirin resistance in coronary syndrome, in this issue of Current Opinion in Pharmacology).

Novel anticoagulants

In comparison to oral Vitamin K antagonist, either direct inhibitors of thrombin or Factors Xa have overall favorable pharmacological effects. Examples of direct Factor Xa inhibitors include apixaban, rivaroxaban, otamixaban, betrixaban, and edoxaban. Direct thrombin inhibitors (Factor IIa inhibitors) were developed with the limitations of standard heparin and warfarin in mind. Examples include ximelagatran, argatroban, and dabigatran etexilate. In common with these novel anticoagulants is the convenience of use with no requirement for laboratory monitoring and limited drug interactions, which may provide multifaceted treatment options for atherosclerosis and anticoagulation in the future [13]. Because of the data available, this review discusses the two anti-Factor X inhibitors, rivaroxaban and apixaban, and the

Table 1

Pharmacological properties of the new oral anticoagulants

	Rivaroxaban	Dabigatran	Apixaban
Target molecule	Factor Xa inhibitor	Factor IIa inhibitor	Factor Xa inhibitor
Prodrug	No	Yes	No
Bioavailability (%)	80–100	~3–8	~52
Maximal plasma concentration (C_{max}) (hour)	2–4	0.5–2	1–3
Potential drug interactions	Potent inhibitors of CYP3A4 and P-gp	Quinidine, amiodarone, potent P-gp inhibitors	Potent CYP3A4 inhibitors
Food interaction	None	None	None
Half-life time (hour)	7–13	11–14	~12
Renal elimination (%)	~33	~85	~27

CYP3A4: cytochrome-P-450 3A4; P-gp: P-glycoprotein.

oral thrombin inhibitor dabigatran etexilate (Table 1) [19••,20–22]. There are differences between Factor Xa and thrombin that may cause these clotting factors to be affected differently by drugs that inhibit them. Currently, the only known functions of Factor Xa are promotion of coagulation and inflammation [23]. Thrombin has more diverse actions in the body; in addition to its known effects on coagulation and inflammation, thrombin also activates protein C (which has anticoagulant properties) and promotes cellular proliferation.

Rivaroxaban

Rivaroxaban is a direct Factor Xa inhibitor with high plasma protein binding (92–95%). In a concentration-dependent manner, rivaroxaban inhibits free Factor Xa and prothrombinase-bound and clot-associated Factor Xa. As a consequence, rivaroxaban prevents thrombin generation by inhibiting Factor Xa generated via both the intrinsic and extrinsic coagulation pathways [4] but does not exhibit direct effect on platelet aggregation induced by collagen, adenosine diphosphate or thrombin [24].

The pharmacokinetic profile of rivaroxaban shows favorable safety and tolerability profile. The bioavailability of a 10 mg dose of rivaroxaban is high (80–100% when combined with meal), and rivaroxaban is rapidly absorbed, reaching a maximal plasma concentration (C_{max}) within two to four hours after oral administration (Table 1). Rivaroxaban displays linear pharmacokinetics, with a half-life of 7–11 hours in young subjects and 11–13 hours in elderly subjects and no significant accumulation after repeat dosing. Absorption is unaffected by food or antacids. Furthermore, there is no inhibition or induction of cytochrome-P-450 (CYPP450) isoforms. Although rivaroxaban is metabolized by CYP3A4, clinically significant drug–drug interactions are only expected with strong CYP3A4 inhibitors or inducers. Rivaroxaban is eliminated in two-thirds through metabolic degradation in the liver, half of which is excreted via the kidneys and half via the hepatobiliary route. One-third of the dose is eliminated as unchanged drug in the urine. There are no active circulating metabolites of rivaroxaban.

Apixaban

Apixaban, like rivaroxaban, interrupts the coagulation cascade by blocking the enzymatic activity of Factor Xa [25,26]. Apixaban is a direct inhibitor of Factor Xa. Orally administered apixaban reaches a bioavailability of 50% and C_{max} is three to four hours (Table 1). The half-life is 10–14 hours after repeated doses. Apixaban is metabolized in part by CYP3A4; it is partly eliminated by the kidneys (25%) and, to some extent, also processed via CYP-independent mechanisms in the liver. Similarly to rivaroxaban, apixaban does not induce or inhibit CYP enzymes and has a low likelihood of clinically significant drug–drug or food–drug interactions.

Dabigatran etexilate

Dabigatran directly inhibits both free and clot-bound thrombin [13••]. After oral administration the prodrug dabigatran etexilate is rapidly converted to dabigatran in the liver, with C_{max} of 1.5 hours after oral ingestion (Table 1). Dabigatran has a half-life of 14–17 hours at steady state. In contrast to rivaroxaban, bioavailability is low with only 7.2%, and therefore it is predominantly excreted in the feces. Although part of the bioconversion from prodrug to active metabolite occurs in the liver, the CYPP450 system is not involved. As dabigatran is a substrate of P-glycoprotein, potentially important drug interactions with the P-glycoprotein inhibitors quinine/quinidine and verapamil have been described. After hepatic activation, 80% of dabigatran is eliminated in the kidneys; thus, as for rivaroxaban and apixaban, patients with severe renal impairment have been excluded from most clinical trials.

Clinical data for the novel oral anticoagulants in acute atherothrombosis

All three novel oral anticoagulants have been evaluated in clinical trials for prevention of primary or secondary venous thromboembolism, and nonvalvular atrial fibrillation. Rivaroxaban and arixaban have been investigated for secondary prevention of atherothrombosis following acute coronary syndromes in two phase III trials and dabigatran in a phase II trial. Table 2 compares the results

Table 2

Ischemic and bleeding event rates in drug trials for peripheral arterial disease and acute coronary syndrome

Events (%)	Peripheral arterial disease		Coronary artery disease with acute coronary syndrome			
	WAVE		APPRAISE-2		ATLAS ACS 2-TIMI 51	
	ASA	ASA + warfarin	Apixaban	Placebo	Rivaroxaban (2.5 mg)	Placebo
Death, MI, stroke	13.3	12.2	8.8	8.9	9.1	10.7
Death MI, stroke, limb ischemia	17.4	15.9				
Cardiovascular death	6	6.1	2.8	3.0	2.7	4.1
Major bleeding	[a]2.2	[a]6.9	1.3	0.5	1.8	0.6
Fatal bleeding	0.3	0.9	0.1	0	0.1	0.2
Net events	19.6	22.8	10.1	9.4	10.9	11.3
Net deaths	6.3	7.0	2.9	3.0	2.8	4.3

WAVE denotes Warfarin Antiplatelet Vascular Evaluation (NCT00125671), mean follow-up 35 months (Ref. [8••]).
APPRAISE-2 denotes Apixaban for Prevention of Acute Ischemic Events 2 (NCT00831441), mean follow-up 8 months (Ref. [31]).
ATLAS ACS2-TIMI 51 denotes Anti-Xa Therapy to Lower Cardiovascular Events in Addition to Standard Therapy in Subjects with Acute Coronary Syndrome — Thrombolysis in Myocardial Infarction 51 (NCT00809965), mean follow-up 13 months (Ref. [9]).
In APPRAISE-2 and ATLAS ACS2-TIMI 51 standard therapy was aspirin with 81% and 93% that were on thienopyridine at time of randomization.
[a] Includes moderate bleedings. Life-threatening bleeding and fatal bleeding is 4.9% for combined therapy and 1.5% for antiplatelet therapy.

of the two phase III trials with the novel anticoagulants in acute coronary syndrome and of warfarin with aspirin in patients with PAD.

Clinical data on rivaroxaban

Rivaroxaban has been approved not only for the prevention of primary or recurrent venous thromboembolism but also for prevention of systemic embolization in nonvalvular atrial fibrillation [27–29]. However, the indication for prevention of recurrence of atherothrombosis was rejected in July 2012 by the Food and Drug Administration due to increased bleeding risk in patients with a recent acute coronary syndrome although rivaroxaban reduced the risk of the composite end point of death from cardiovascular causes, myocardial infarction, or stroke [19••]. Rivaroxaban increased the risk of major bleeding and intracranial hemorrhage but not the risk of fatal bleeding. In the ATLAS ACS 2-TIMI 51 trial, a double-blind, placebo-controlled trial published early in 2012, approximately 15 000 patients with a recent acute coronary syndrome received twice-daily doses of either 2.5 mg or 5 mg of rivaroxaban or placebo in addition to aspirin which in 81% was combined with clopidogrel for a mean of 13 months. The trials' primary efficacy end point was a composite of death from cardiovascular causes, myocardial infarction, or stroke. An event rate of 8.9% was observed for rivaroxaban and 10.7% for placebo (hazard ratio 0.84; 95% confidence interval, 0.74–0.96; $P = 0.008$), with significant event reduction for both the twice-daily 2.5-mg dose (9.1% versus 10.7%, $P = 0.02$) and the twice-daily 5-mg dose (8.8% versus 10.7%, $P = 0.03$). Remarkably, the twice-daily 2.5-mg dose of rivaroxaban reduced the rates of death from cardiovascular causes (2.7% versus 4.1%, $P = 0.002$) and from any cause (2.9% versus 4.5%, $P = 0.002$). In contrast, this survival benefit that was not achieved with the twice-daily 5-mg dose. As compared with placebo, rivaroxaban increased the rates of major bleeding (2.1% versus 0.6%, $P < 0.001$) and intracranial hemorrhage (0.6% versus 0.2%, $P = 0.009$), without a significant increase in fatal bleeding (0.3% versus 0.2%, $P = 0.66$) or other adverse events. The twice-daily 2.5-mg dose resulted in fewer fatal bleeding events than the twice-daily 5-mg dose (0.1% versus 0.4%, $P = 0.04$).

The striking finding of the ATLAS ACS 2-TIMI 51 trial is that the survival benefit for rivaroxaban was only observed with the 2.5-mg twice-daily dose, one quarter of the dose studied in atrial fibrillation or venous thromboembolism, but again suggesting that higher doses may offset the benefit with more bleeding events.

Clinical data on apixaban

The APPRAISE-2 trial (apixaban after acute coronary syndromes) contrasts the ATLAS ACS 2-TIMI 51 trial since it was stopped early due to a highly significant increase in major bleeding without any relevant reduction in ischemic events [30••]. Only after a median follow-up of 241 days, the primary outcome of cardiovascular death, myocardial infarction, or ischemic stroke occurred in 7.5% of the patients assigned to apixaban and in 7.9% assigned to placebo (hazard ratio with apixaban, 0.95; 95% confidence interval, 0.80–1.11; $P = 0.51$). The primary safety outcome of major bleeding occurred in 1.3% of the patients who received apixaban and in 0.5% with placebo (hazard ratio with apixaban, 2.59; 95% confidence interval, 1.50–4.46; $P = 0.001$). A greater number of intracranial and fatal bleeding events occurred with apixaban than with placebo (0.3/0.1% versus 0.1/0%).

Clinical data on dabigatran etexilate

There is only a dose findings study (phase II) for the oral direct thrombin inhibitor dabigatran etexilate (RE-DEEM) [31–33]. In the RE-DEEM trial, with almost all patients receiving dual platelet inhibition, a

dose-dependent increase in clinically relevant bleeding events was observed, with highest rates with the dabigatran etexilate 110 mg and 150 mg twice daily as currently used in atrial fibrillation. The most frequently reported bleeding events were gastrointestinal bleeding and epistaxis. Although the study was not powered to demonstrate efficacy in cardiovascular death, nonfatal myocardial infarction or nonhemorrhagic stroke, lower event rates was attained in the 2 higher dabigatran doses (110 mg twice daily, 3.0%; 150 mg twice daily, 3.5%) compared with the lower doses (50 mg twice daily, 4.6%; 75 mg twice daily, 4.9%) and the placebo group (3.8%).

Clinical data on other emerging anticoagulants

Darexaban, another Factor IIa Inhibitor, was evaluated for safety and tolerability for the prevention of ischemic events in acute coronary syndromes [34]. Darexaban, when added to dual antiplatelet therapy, produced a dose-related twofold to fourfold increase in bleeding versus placebo, with no other safety concerns, but also with no efficacy.

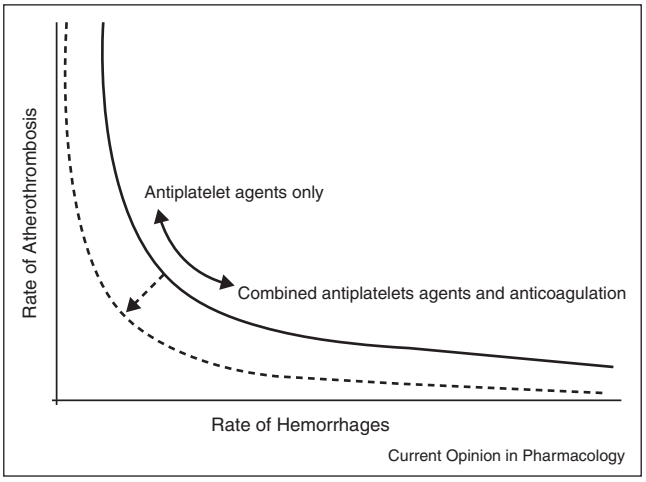
Discussion

Two phase III studies were conducted for secondary prevention of atherothrombotic events with rivaroxaban and apixaban (the APPRAISE-2 trial and ATLAS ACS 2-TIMI 51) — of which the APPRAISE was prematurely terminated because of an excess of bleeding with apixaban and no evidence of benefit [19••,30••]. No phase III trial was conducted with dabigatran because of similar safety issues following the phase II study and because of results suggesting an increased risk of myocardial ischemia [33]. Only the ATLAS ACS 2-TIMI 51 not only met its primary objective but also a threefold to fourfold increase in intracranial bleedings [31].

This differences in net event outcome between APPRAISE-2 and ATLAS ACS 2-TIMI 51 are not well explained by different bleeding rates, which were similar in both trials. The APPRAISE-2 population was older and more commonly, had diabetes, renal insufficiency, and history of stroke, and more severe myocardial ischemia [32]. There was no significant benefit in efficacy outcome for death, myocardial infarction, or stroke in APPRAISE-2 (Table 2). Interestingly, FXa inhibition potency of the studied doses was different: APPRAISE-2 used the same 5 mg twice daily apixaban dose tested in atrial fibrillation, whereas ATLAS-2 used 2 doses (2.5 mg/5 mg twice daily), that were one fourth to one half of the total daily dose of rivaroxaban (20 mg once daily) tested in atrial fibrillation. This might hypothetically suggest that a lower, rather than a higher level of FXa inhibition per se may have a better antithrombotic effect.

Taken together, the combination of a dual antiplatelets therapy with a novel oral anticoagulant is associated with dose-dependent increased risk for major and intracranial bleeding risk and only with rivaroxaban with a significantly lower rate of ischemic related deaths. Although the American Food and Drug Administration did not approve rivaroxaban for secondary prevention of acute coronary syndrome, ATLAS ACS 2-TIMI 51 provides evidence that reduction of the persistently high morbidity and mortality after myocardial ischemia is possible (Figure 2). Combination of antithrombotic therapy shifts the ischemic-bleeding balance in a hyperbolic function with slightly lower recurrent atherothrombosis but higher risk for major bleeding, or vice versa if no anticoagulant is added. Ideally, novel antithrombotic drug should decrease recurrent atherothrombotic events without increasing major bleedings. Nevertheless, the combination of only one antiplatelet agent (aspirin) with one new oral anticoagulant — of which rivaroxaban at a dosage of 2.5 mg twice daily seems to be the candidate — might be tempting for patient with PAD. In contrast to patients with a recent acute coronary syndrome, patients with PAD at a chronic stage are not treated by dual antiplatelets drugs irrespective of their risk for atherothrombotic events. As mentioned above, PAD patients are an atherosclerotic high risk group and there are no trials for PAD patients evaluating the role of combined anticoagulant therapy after acute events. However, due to the increased risk in PAD, a logic consequence would be a trial — similar to the WAVE study (warfarin and aspirin) — with aspirin and low dose rivaroxaban [8••]. With regard to the high incidence of atherothrombotic events in this atherosclerotic subgroup, an improved therapy for secondary prevention is still

Figure 2



Schematic illustration of ischemia and bleeding risk in atherosclerosis in dependence of antithrombotic regimen. The assumed hyperbolic function indicates that in relation to antithrombotic regimen the risk shifts either toward higher bleeding or higher ischemic risk. Ideally, the novel antithrombotic drugs should shift the risk toward both lower ischemic and bleeding rates (dotted line).

needed for PAD. The main safety issue with bleeding risk will jeopardize the potential antithrombotic benefit, and patients with PAD are usually older and more fragile. Hence, PAD patients with previous history of cerebral ischemia or major bleedings should be evaluated with great caution. In contrast to WAVE trial with warfarin and a target international normalized ratio of 2.0–3.0, a trial with rivaroxaban 2.5 mg combined with aspirin represents a lower bleeding risk, since 2.5 mg of rivaroxaban is a quarter of the dosage that is used for atrial fibrillation.

In conclusion, atherothrombosis is often insufficiently controlled by antiplatelet therapy alone. Combination of antiplatelet agents with novel anticoagulants may reduce ischemic events but is associated with an increased risk for major bleedings. So far, no novel anticoagulant drug is approved for secondary prevention following acute coronary syndrome. PAD patients are a fragile subgroup of atherosclerotic patients with substantial risk for atherothrombotic events and need to be carefully evaluated for combined antithrombotic therapy with the novel anticoagulants.

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